Pollen morphology of Iranian species of *Typha* (Typhaceae) and its taxonomic significance

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Abstract

Pollen analyses of 12 Iranian species of *Typha* were conducted through Light Microscopy (LM) and Scanning Electron Microscopy (SEM). Among the 12 studied species, we found that 5 species, *T. caspica, T. latifolia*, *T. lugdunensis*, *T. minima* and *T. shuttleworthii*, present pollen in tetrads, whereas the remaining 7 species, *T. angustifolia, T. azerbaijanensis*, *T. domingensis*, *T. grossheimii*, *T. kalatensis, T. laxmanii* and *T. tourcomanica*, have pollen in monads. The exine of *Typha* species is mostly reticulate although a few species present perforate-microreticulate and rugulate exine. Our analyses revealed that using a combination of LM and SEM characters the identification of species is feasible. A diagnostic key based on palynological traits is presented for all studied taxa.

Key words: monad, morphology, pollen, palynology, Tetrad, *Typha*, Typhaceae, Iran

Introduction

Cattails, genus *Typha* L. (Typhaceae) is distributed throughout all continents except Antarctica, showing the most diversity in South-West and Central parts of Asia (Kronfeld, 1889). It includes some 24 species that are perennial herbs that grow in a variety of aquatic and semi-aquatic habitats (Parsa 1950, Riedl 1970, Davis 1982 Kronfeld 1889, Mavrodiev 2002, Kim *et al*. 2003, Hamdi & Assadi 2003). The most characteristic morphological trait is the presence of cylindrical inflorescences with he staminate flowers positioned above pistillate flowers along the inflorescence axis. *Typha* species are one of the most conspicuous members of the shallow water communities of the world. Species of *Typha* readily expand to form monospecific stands because of the profusely growing rhizome that excludes other plants (Graze & Wetzel 1981).

During the course of a revision of *Typha* for the Flora of Iran, twelve species were recognized in the area (Hamdi & Assadi 2003). These included some widespread cosmopolitan species such as, *T. angustifolia* L., *T. latifolia* L., and *T. domingensis* Pers., some Euro-Mediterranean and Euro-Asiatic species such as, *T. caspica* Pobed, *T. grossheimii* Pobed, *T. laxmannii* Lepechin, *T. lugdunensis* Chab., *T. minima* Funk, *T. shuttleworthii* Koch. & Sonder *T. turcomanica* Pobed, as well as two endemics such as, *T. azerbaijanensis* Hamdi & Assadi and *T. kalatensis* Assadi & Hamdi,

The taxonomy and systematics of *Typha* has remained unclear and controversial. This is mostly the result of species being very similar in overall gross morphology and the large variability in vegetative and reproductive traits used to delimit the taxa (Smith, 1967). Since early systematic treatment of Kronfeld (1889), several revisions have been conducted to establish taxonomic boundaries between taxa based on morphological traits at different regional scales including: Australia (Finlayson *et al.* 1985), Europe (Cook 1980), India (Saha 1968), Iran and Pakistan (Bokhari 1983, Hamdi & Assadi 2003), Korea and eastern Russia (Kim *et al.* 2003), North America (Kuehn & White 1999), or have been focused to study particular species complexes (Kuehn *et al*. 1999) or to investigate their taxonomic identity (Sharma & Gopal 1980).

High morphological similarity among taxa is a clear indication of their little genomic differentiation which results in their high potential to produce interspecific hybrids (Smith, 1967, Kuehn *et al.* 1999) that may blur morphological differences between different species in sympatric populations. Besides, environmental variation can contribute to increase the lability of vegetative and inflorescence traits that have been traditionally used for species identification. Molecular markers have been evaluated as a potentially neutral source of information to investigate genetic differentiation between populations and species, to assist species identification and to establish taxonomic relationships. These studies have been mostly focused on the more widespread species-pair, *T. latifolia-T.angustifolia* (Keane *et al.* 1999, Lamote *et al.* 2005) and their putative hybrid *T. glauca* Godr. (Kuehn *et al.* 1999). Despite, *Typha* has not been extensively studied with molecular markers and that the identification of species-specific molecular markers on which base their taxonomic recognition will be very promising, molecular studies still depend on *a priori* reliably morphologically identified samples (Kuehn & White 1999). This suggests that the exploration of further morphological sources of information and the evaluation of their taxonomically informative potential is still required in *Typha*. Unexplored morphological characters could provide an enlarged data set of traits in which base a solid taxonomy and systematics for the genus. Accordingly, stigmatic characters were identified as a potentially taxonomically useful (Kuehn & White, 1999) but their use is restricted to mature flowering plants.

The taxonomic and systematic potential of micromorphological data derived from pollen has not been extensively surveyed in the Typhaceae. Palynological data in *Typha* are mostly restricted to comparative microsporogenesis studies including a limited sampling of taxa. Those studies showed that successive microsporogenesis occurs in *Typha* as for many Monocotyledon families (Svarkala & Larson 1963, Furness & Rudall 1999). However, micromorphological traits have not been investigated in a sufficient species to reveal their usefulness in taxonomy and systematics. Nonetheless, recently Finkelstein (2003) used palynological data to investigate the range expansion of *T. angustifolia* and *T. glauca* from pollen stratigraphies in North America and revealed their likely use for species identification.

In this study we investigate pollen micromorphological characters from 12 species of *Typha* from Iran and evaluate their usefulness in establishing the taxonomical relationships of the studied species.

Material and methods

Pollen grains of all 12 species of the genus *Typha* occurring in Iran were studied using both Light Microscopy (LM) and Scanning Electron Microscopy (SEM). The pollen samples were obtained mostly from freshly collected samples and herbarium vouchers deposited at TARI (acronym according to Holmgren *et al*. 1998), see Appendix. For LM analysis, fresh pollen grains were acetolyzed following the standard method described by Erdtman (1969), mounted on glycerine jelly glass slides, observed under a BX50 light microscope and measured using a micrometer to the nearest 0.05µm. Twelve pollen grains were measured from each sampled population. Samples for SEM analyses were prepared according to Davies (1999) with minor modifications. Pollen grains were mounted on 12.5mm diameter stubs and attached with sticky tape and then sputter-coated with approximately 25µm of Gold-Paladium. The pollen samples were examined and photographed using a LEO-440I Scanning Electron Microscope, set at an accelerating voltage of 10–15 kV. Cross-section of exine was also examined. For each pollen grain polar (*P*) and equatorial (*E*) diameters were measured and the P/E ratio was calculated so that the degree of asymmetry could be estimated. The length of the lumina (*L*) and the length (L_m) and width (W_m) of the longest and widest muri on distal polar surface were measured from SEM preparations. The terminology used for describing pollen ornamentation followed Moore *et al*. (1991), Kosenko (1999), Punt *et al*. (1994, 2007) and Schols *et al*. (2003) .

Results and discussion

A summary of the main morphological characters of the pollen from the 12 Iranian species of *Typha* is presented in Table 1. Our investigation showed that two basic types of pollen can be found in the Iranian species of *Typha*. These two types are related to the type of pollen dispersal in *Typha* which can be as tetragonal tetrads (Figs. 1-2 and 3A-C) or as monads (Figs. 3D-F, 4-6). Tetrad pollen is found in five Iranian species: *T. caspica, T. latifolia*, *T. lugdunensis*, *T. minima* and *T. shuttleworthii*, whereas monad pollen is found in the seven remaining ones: *T. angustifolia, T. azerbaijanensis*, *T. domingensis*, *T. grossheimii*, *T. kalatensis, T. laxmanii* and *T. tourcomanica*. Pollen size ($P \times E$) ranged from the smallest size of *T. angustifolia* (9.40-15.93µm × 14.65-24.17µm) to the maximum scored for *T. laxmanii* (22.13-34.38µm × 24.10-34.85µm). P/E ratios ranged from 0.54 to 0.96 (oblate-spheroidal to oblate). The aperture system is ulcerate (Furness & Rudall 1999) with a single pore, i.e. pollen monoporate (Fig. 4A). Exine microsculpturing differed among species of *Typha*. This was perforate-microreticulate in single species, *T. laxmanii* (Fig. 4A-C). Two species, *T. caspica* and *T. latifolia* had perforate-reticulate exine (Fig. 2). Six species showed reticulate exine, which was the most common microsculpture pattern in *Typha* (Table 1, Figs. 3C-F, 4C-F, 5 and 6). The remaining 3 species, *T. lugdunensis*, *T. minima* and *T. shuttleworthii*, showed rugulate exines (Figs. 1 and 3A-C).

Analysis of tecta microsculpturing related to the length of lumina and length and width of muri also revealed differences between species of *Typha*. The smaller lumina (0.1-0.3µm) corresponded to *T. azerbaijanensis* and *T. caspica*, (Figs. 2A-C, 4D-F), whereas the largest lumina (0.7-1.9µm) corresponded to *T. angustifolia* (Fig. 3-D-F) and did not correspond to the class of general microsculpturing pattern they were classified. Similarly, size of muri varied from a smallest value in *T. minima* (0.8-1.0×0.3-0.4µm) and *T. lugdunensis* (1.0-1.8×0.2-0.4µm), respectively (Fig. 1, Table 1) to a largest value (2.0-3.0×0.3-1.0µm) in *T. latifolia* (Fig. 2D-F, Table 1). The distribution of all these microstructural traits, whereas useful for the species differentiation did not allow to interpret natural groups within the genus according to previous classifications.

Considering all the micromorphological data presented in this paper, we found that the most remarkable trait grouping the different studied species of Typha is related to the mode in which pollen is released (i.e. monads or tetrads). While in this study no intermediate forms between both types were found, species with tetrad triad, diad and monad pollen have been reported elsewhere, i.e. *T. glauca* (Finkelstein, 2003)- This species is not present in the Iranian flora and thus, was not included in this study however, this result still does not invalidate the systematic value of this trait since this species was proposed to be a hybrid between *T. latifolia*, a species with tetrad pollen and *T. angustifolia*, a species with pollen in monads (Kuehn *et al*. 1999).

The differentiation of these two basic types contrasts with the systematic treatment of Kronfeld (1889) that recognised species of *Typha* into two sections depending on the presence (Section *Bracteolatae*) or absence (Section *Ebracteolatae*) of bracteoles in the female flowers. Other vegetative and reproductive morphological traits investigated in *Typha* also did not agree with the mentioned sectional arrangement of the genus (Kim *et al*. 2003). Whether each of the groups found here according to pollen morphology or those proposed earlier by Kronfeld (1889) on flower morphology may constitute monophyletic groups, should be tested on a phylogenetic basis.

According to our results we present an identification key to the 12 Iranian species of *Typha* based on LM and SEM palynological characters:

1a. Pollen in tetrads..2
2a. Exine perforate-reticulate...3
3a. Polar length shorter than 22µm, lumina narrower than 0.3µm.....................*T. caspica*
3b. Polar length longer than 23µm, lumina wider than 0.6µm...........................*T. latifolia*
2b. Exine rugulate...4
4a. P/E ratio larger than 0.8...*T. minima*
4b. P/E ratio smaller than 0.7..5
5a. P/E ratio 0.6-0.7, muri longer than 2µm...............................*T. shuttleworthii*
5b. P/E ratio up to 0.60, muri shorter than 1.9µm.........................*T. lugdunensis*
1b. Pollen in monads..6
6a. P/E ratio larger than 0.9...7
7a. Mean polar length longer than 25µm, exine perforate-microreticulate........*T. laxmanii*
7b. Mean polar length shorter than 22µm, exine reticulate...8
8a. Lumina narrower than 0.5 µm, muri longer than 2.5µm.......*T. tourcomanica*
8b. Lumina 0.6µm or wider, muri up to 2.0µm long......................*T. grossheimii*
6b. P/E ratio smaller than 0.8...9
9a. Mean polar length longer than 18µm, lumina up to 0.2µm wide......*T. azerbaijanensis*
9b. Mean polar length up to 17µm, lumina 0.3µm or wider...10
10a. Mean ecuatorial length up to 22µm..*T. angustifolia*
10b. Mean ecualorial length longer than 25µm...11
11a. P/E ratio up to 0.55, lumina 0.8µm or wider, muri longer than 2.0µm...*T. kalatensis*
11b. P/E ratio higher than 0.6, lumina narrower than 0.6µm, muri up to 1.2µm long... *T. domingensis*

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APPENDIX

Representative materials examined

Typha angustifolia
Iran: Prov. Tehran, 0 m, 20.iv.2000, *Hamdi 80884* (TARI).

Typha azerbaijanensis
Iran: Prov. Azerbaijan, Khoy, toward Marand, margin of road, 1100 m, 12.vi.2000, *Hamdi 81266*(TARI).

Typha caspica
Iran: Prov. Guilan, Bandare-Astara, 0 m, 5.vi.2000, *Hamdi 80869*(TARI).

Typha domigensis
Iran: Prov. Azerbaijan, Parsabad, Pirovatloo, 70 m, 3.vi.2000, *Hamdi 80887*(TARI).

Typha grossheimii
Iran: Prov. Azerbaijan, Parsabad, 100 m, 12.vi.1995, *Mozaffarian 64257*(TARI).

Typha kalatensis
Iran: Prov. Khorrasan, 15 km Bojnourd to Shirvan, Babaaman, 1200 m, 22.iv.2000, *Hamdi 80885*(TARI).

Typha latifolia
Iran: Prov. Mazandaran, 5 km Kourdkouy toward Bandare-Turkaman, 150 m, 14.v.2000, *Hamdi 80853*(TARI).

Typha laxmanii
Iran: Prov. Fars, Estahbanat, south of Bash mountain, 1700-2200m, 6.iv.1986, *Mozaffarian & al. 47032* (TARI).

Typha lugdunensis
Iran: Prov. Fars, Firouzkouh, margin of Nemroud River, 1500-1650 m, 15.iv.1998, *Mozaffarian & al. 58975* (TARI).

Typha minima
Iran: Prov. Fars, Firouzkouh, Harandeh village, margin of Nemroud River, 1500-1650 m, 10.iv.1999, *Hamdi 80845* (TARI).

Typha shuttleworthii
Iran: Prov. Azerbaijan, Oshnavieh, 50 km towards Uromieh, margin of road, 1750 m, 13.vii.1992, *M. Assadi, 78892*(TARI).

Typha tourcomanica

Iran: Prov. Mazandaran, Kourdkoy toward Bandare- Turkaman, margin of road 150 m, 14.v.2000, *Hamdi 80886*(TARI).

Table 1. Quantitative and qualitative traits of pollen grains in Iranian species of *Typha*. *N* = 12 measurements.

	P		***E***							
Species	**Mean±SD**	**Range**	**Mean±SD**	**Range**	***P/E***	***L***	L_m	W_m	***Pollen presentation***	***Exine ornamentation***
T. angustifolia	12.47±1.75	9.40-15.93	20.26±3.10	14.65-24.17	0.62	0.7-1.9	2.0-2.2	0.5-0.7	Monads	reticulate
T. azerbaijanensis	20.17±3.09	15.36-26.18	26.93±1.79	24.00-29.65	0.75	0.1-0.2	1.9-2.1	0.3-0.6	Monads	reticulate
T. caspica	18.74±1.74	15.50-22.00	25.76±2.31	21.24-31.45	0.73	0.1-0.3	2.5-3.0	0.6-1.0	Tetrads	Perforate-reticulate
T. domigensis	16.62±2.72	13.51-22.38	25.75±2.12	22.00-29.50	0.65	0.3-0.6	1.0-1.2	0.6-0.8	Monads	reticulate
T. grossheimii	20.09±2.89	16.50-25.84	21.01±2.56	18.43-23.58	0.96	0.6-0.8	1.6-1.8	0.8-1.0	Monads	reticulate
T. kalatensis	14.56±2.52	11.25-19.18	26.94±3.15	21.57-32.83	0.54	0.8-1.5	2.0-2.4	0.3-0.6	Monads	reticulate
T. laxmanii	27.06±3.44	22.13-34.38	29.39±3.03	24.10-34.85	0.92	0.5-1.1	3.5-4.0	0.6-0.9	Monads	Perforate-microreticulate
T. latifolia	25.70±1.58	23.21-27.80	30.31±2.44	25.20-34.22	0.85	0.6-1.2	2.0-3.0	0.3-1.0	Tetrads	Perforate-reticulate
T. lugdunensis	15.77±3.10	11.18-21.47	26.47±3.54	19.35-34.13	0.60	0.4-1.0	1.0-1.8	0.2-0.4	Tetrads	rugulate
T. minima	18.55±3.13	15.25-25.13	21.48±2.73	15.23-27.28	0.86	0.3-0.8	0.8-1.0	0.3-0.4	Tetrads	rugulate
T. shuttleworthii	17.02±3.97	12.20-25.20	25.50±2.25	21.23-29.82	0.67	0.3-0.6	2.2-2.7	0.3-1.1	Tetrads	rugulate
T. tourcomanica	20.24±3.07	15.40-26.21	22.50±2.65	16.23-26.81	0.90	0.3-0.5	2.8-3.0	0.5-0.7	Monads	Reticulate

P = polar length (µm); *E* = ecuatorial length (µm); *L* = lumina length (µm); L_m, W_m = length and width of longest and widest muri, respectively on distal surface (µm); SD = Standard deviation.

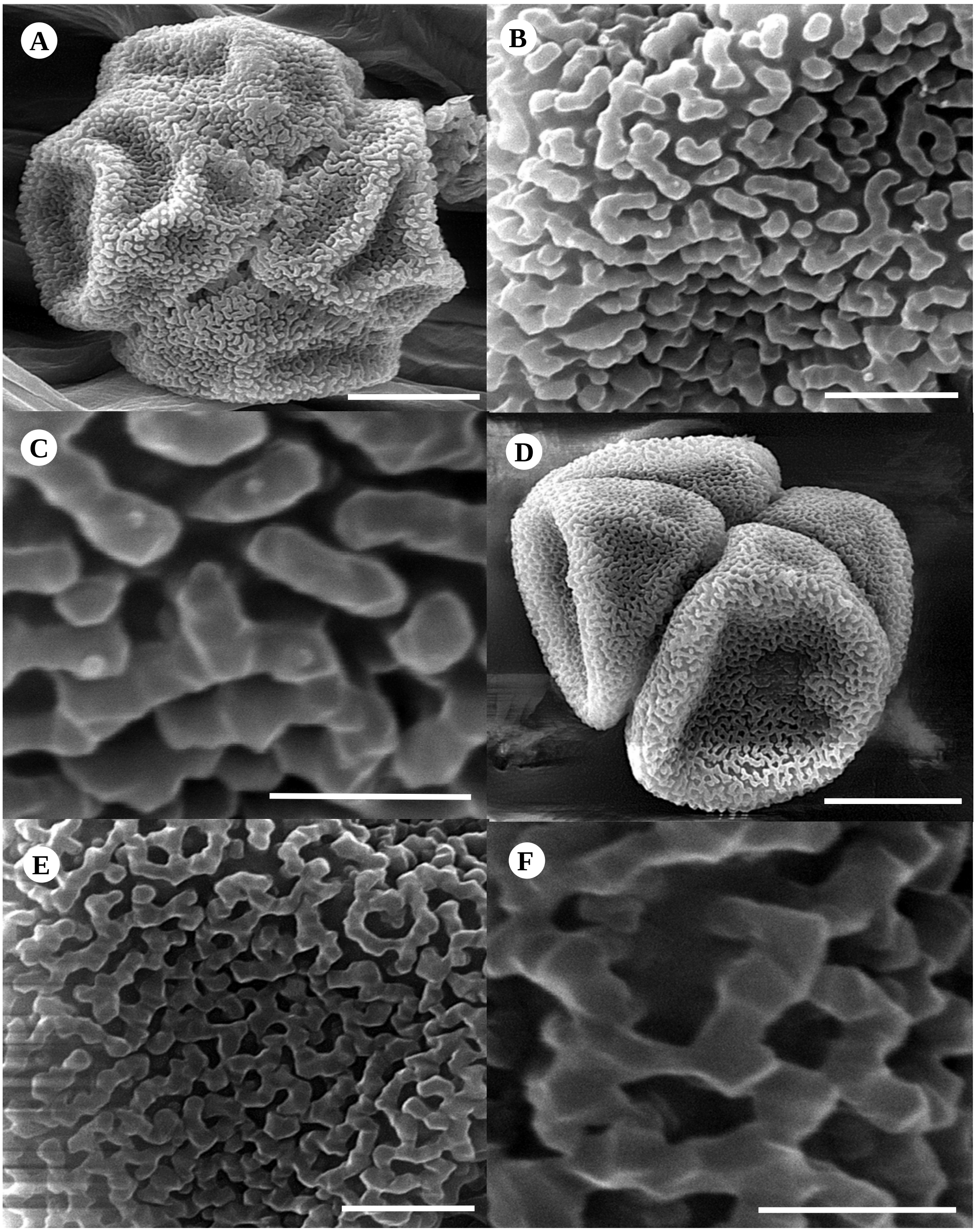

Fig. 1. Scanning Electron Microscopy photographs of pollen of *Typha* species with pollen in tetrads. A-C. *T. minima*. D-F. *T. lugdunensis*. A-F, Distal polar view. B-C, E-F. Detail of the rugulate exine. Scale bars: A, D, 10µm; B, E, 2µm; C, F, 1µm. (A-C, from *Hamdi* 80845 TARI; D-F, from *Mozaffarian et al*. 58975 TARI).

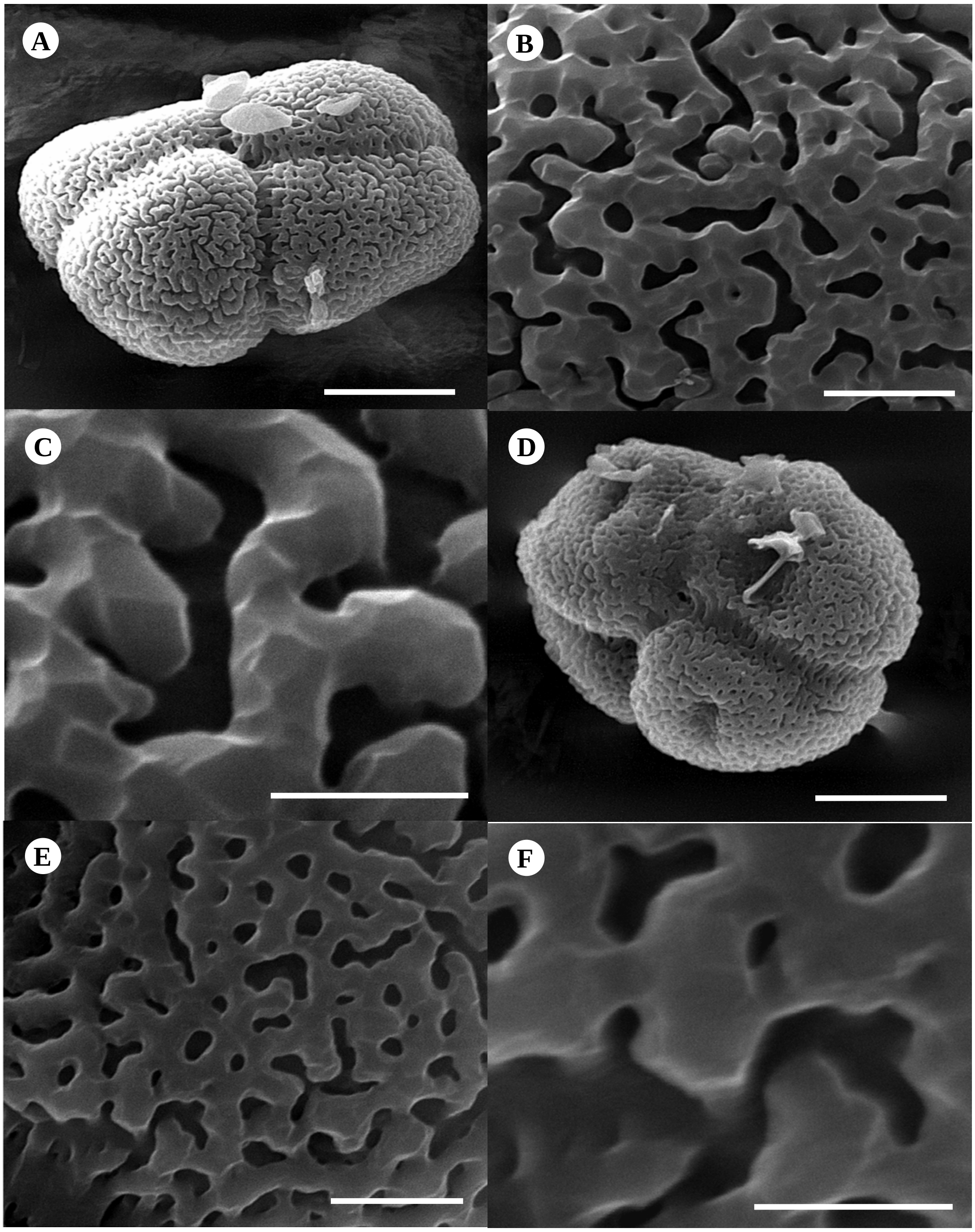

Fig. 2. Scanning Electron Microscopy photographs of pollen of *Typha* species with pollen in tetrads. A-C. *T. caspica*. D-F. *T. latifolia*. A-F, Distal polar view. B-C, E-F. Detail of the perforate-reticulate exine. Scale bars: A, D, 10μm; B, E, 2μm; C, F, 1μm. (A-C, from *Hamdi* 80869 TARI; D-F, from *Hamdi* 80853 TARI).

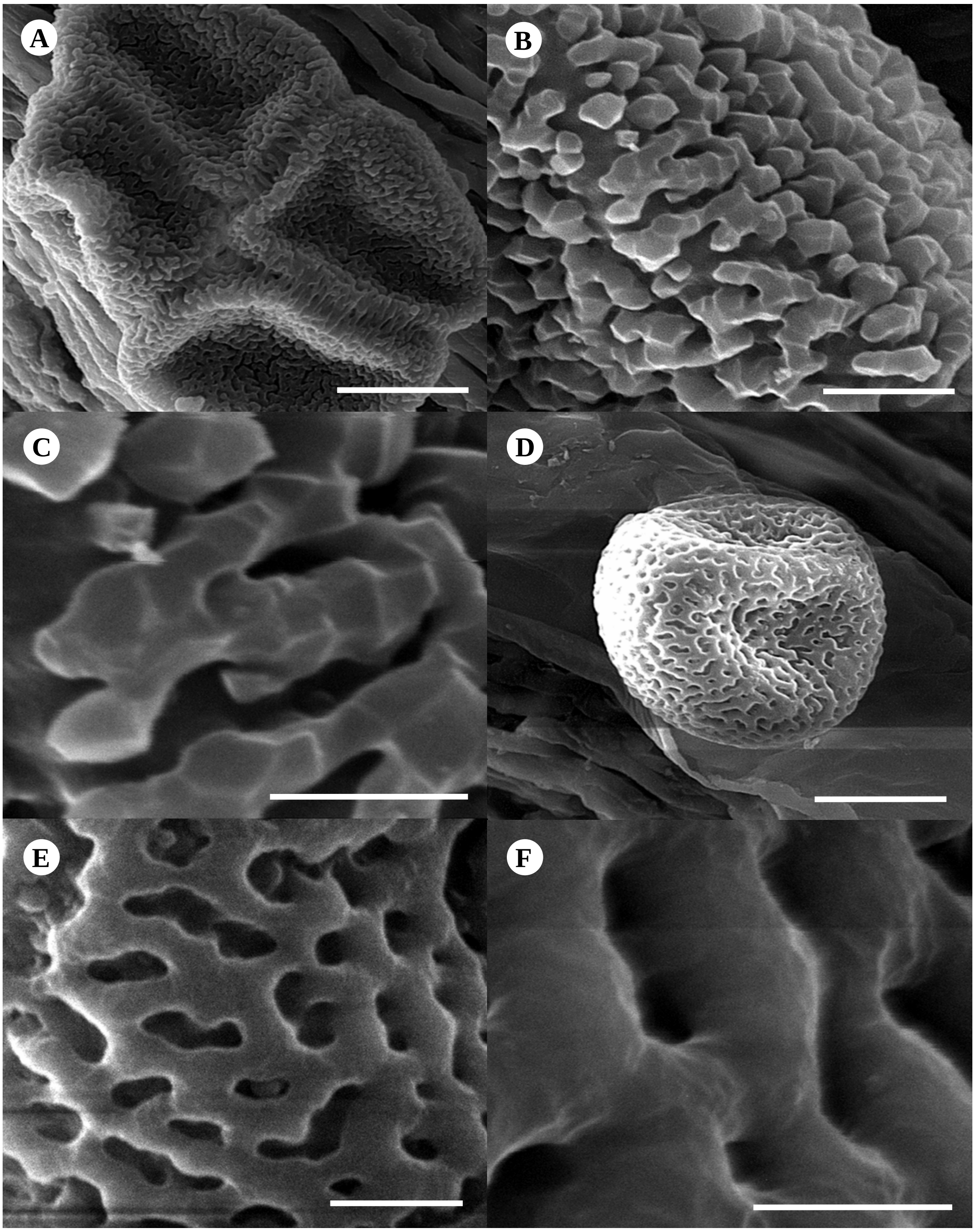

Fig. 3. Scanning Electron Microscopy photographs of pollen of *Typha* species with pollen in tetrads and monads. A-C. *T. shuttleworthii*. D-F. *T. angustifolia*. A-C, Distal polar view. D-F, proximal view. B-C, Detail of the rugulate exine. E-F, Detail of the reticulate exine. Scale bars: A, D, 10µm; B, E, 2µm; C, F, 1µm. (A-C, from *Assadi* 78892 TARI; D-F, from *Hamdi* 80884 TARI).

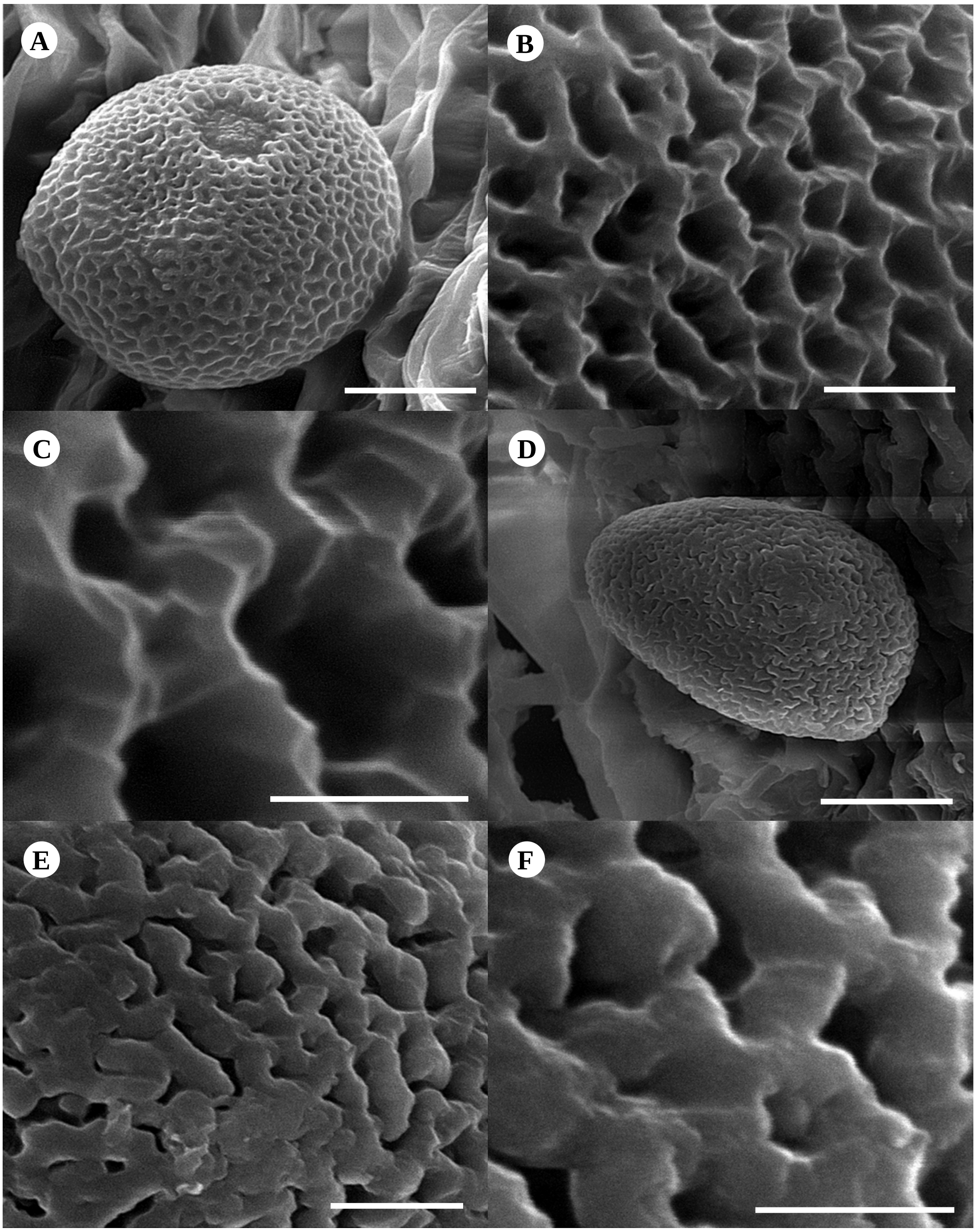

Fig. 4. Scanning Electron Microscopy photographs of pollen of *Typha* species with pollen in monads. A-C. *T. laxmanii*. D-F. *T. azerbaijanensis*. A-C, Distal polar view. D-F, proximal view. B-C, Detail of the perforate-microreticulate exine. E-F. Detail of the reticulate exine. Scale bars: A, D, 10µm; B, E, 2µm; C, F, 1µm. (A-C, from *Mozaffarian* 47032 TARI; D-F, from *Hamdi* 81266 TARI).

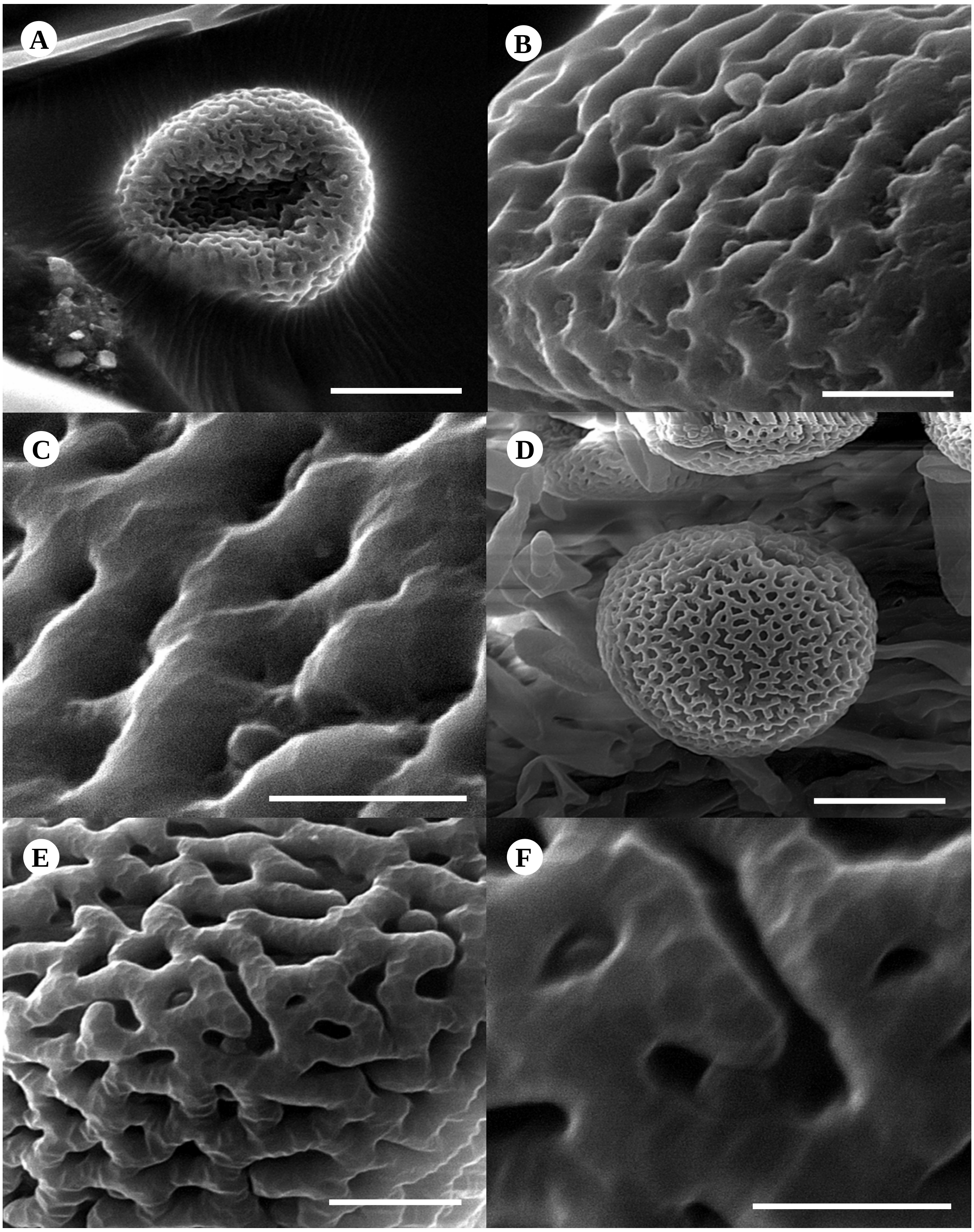

Fig. 5. Scanning Electron Microscopy photographs of pollen of *Typha* species with pollen in monads. A-C. *T. grossheimii*. D-F. *T. tourcomanica*. A-C, Proximal polar view. D-F, Equatorial view. B-C, E-F. Detail of the reticulate exine. Scale bars: A, D, 10μm; B, E, 2μm; C, F, 1μm. (A-C, from *Mozaffarian* 64257 TARI; D-F, from *Hamdi* 80856 TARI).

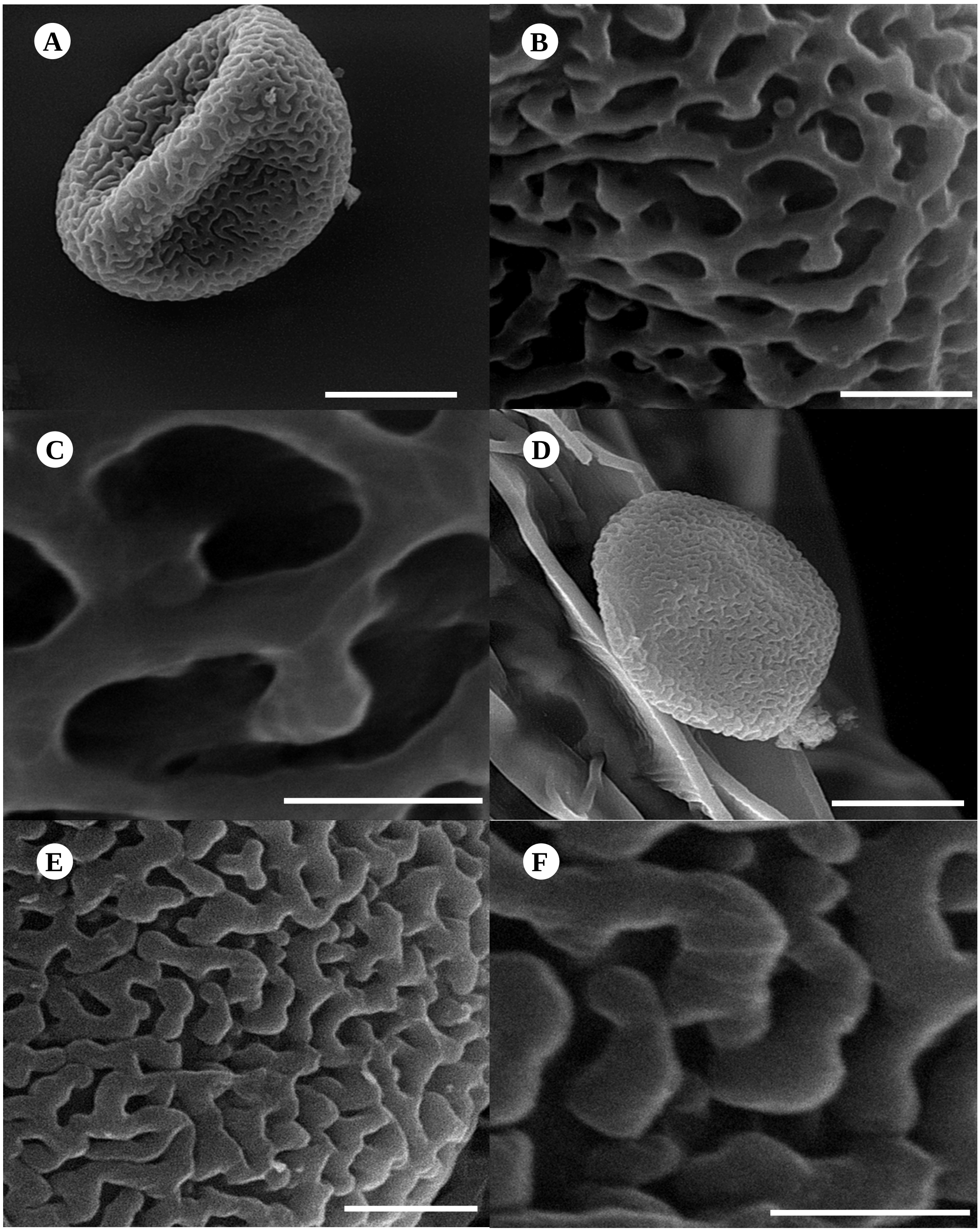

Fig. 6. Scanning Electron Microscopy photographs of pollen of *Typha* species with pollen in monads. A-C. *T. kalatensis*. D-F. *T. domingensis*. A-C, Proximal polar view. D-F, equatorial view. B-C, E-F. Detail of the reticulate exine. Scale bars: A, D, 10µm; B, E, 2µm; C, F, 1µm. (A-C, from *Hamdi* 80885 TARI; D-F, from *Hamdi* 80887 TARI).